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Physical inactivity does not impair the insulin-lowering effects of moderate-intensity exercise, yet it does impair fat metabolism

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Physical inactivity does not impair the insulin-lowering effects of moderate-intensity exercise, yet it does impair fat metabolism

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Abstract

Physical inactivity does not impair the insulin-lowering effects of moderate-intensity exercise, yet it does impair fat metabolism

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Acute exercise and physical activity improve insulin sensitivity, glucose tolerance, and postprandial lipemia, although recent research suggests that physical inactivity may attenuate some of these healthy metabolic benefits of exercise. This study aimed to determine how two days of physical inactivity and physical activity affected exercise-induced changes in plasma insulin, glucose, and triglyceride concentrations during an oral glucose tolerance test (OGTT) performed the next morning. Five untrained men (n=2) and women (n=3) completed three five-day trails in a randomized crossover design. Each trial began with two days of normal activity levels, followed by two intervention days. Two days of physical inactivity (3,666 ± 100 steps) without exercise (SIT) were compared to two days of physical inactivity (3,077 ± 141 steps) with a 1-hr bout of moderate intensity cycling at 65% of VO_{2peak} (SIT + EX). Finally, two intervention days of high activity (12,270 ± 408 steps) were performed with a 1-hour bout of moderate cycling (ACTIVE + EX). The following morning, subjects completed a 120-min OGTT, during which plasma

was collected and analyzed for glucose, insulin, and triglycerides. No changes were observed in plasma glucose. Compared to SIT, insulin total area under the curve (AUC_T) was 39% lower in SIT+EX (p=0.18) and 21% lower in ACTIVE+EX (p=0.48), demonstrating that exercise had an insulin-lowering effect. Plasma triglyceride AUC_T in ACTIVE+EX was 26% lower than SIT (p=0.10) and 28% lower than SIT+EX (p=0.03), and fasting plasma triglyceride concentration in ACTIVE+EX was 34% lower than SIT (p=0.06) and 20% lower than SIT+EX (p=0.43). These data indicate that the insulin-lowering effect of physical activity is influenced to a greater extent by an acute bout of moderate exercise (65% VO_{2peak}) than the background daily steps. Conversely, the triglyceride lowering effect of physical activity appears to be contingent upon higher physical activity (daily step count) and not acute moderate-intensity exercise. This also was the case with postprandial whole-body fat oxidation as ACTIVE+EX was 26% higher when compared to SIT+EX. Taken together, insulin sensitivity appears to be improved by prolonged moderate-intensity exercise (i.e. 65% of VO_{2peak}), while postprandial triglyceride concentration and fat oxidation are improved by increasing daily step count (i.e.; from < 4,000 to >11,000 per day).

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Review of Literature

Introduction

In recent years, diabetes has emerged as a worldwide condition, affecting millions of American lives, and providing a severe financial detriment to the healthcare system. Diagnosed diabetes mellitus affects 34.2 million Americans, representing just over 10% of the national population (CDC, 2020). An additional 88 million American adults suffer from prediabetes, defined as fasting plasma glucose between 100 - 125 mg/dl or an A1C value of 5.7% - 6.4% (CDC, 2020). Combining these findings reveals that well over a third of the entire population of the United States is diabetic or pre-diabetic, and according to the 2020 report from the Center for Disease Control (CDC), both direct and indirect costs of diagnosed diabetes in the United States was $327 billion in 2017 (CDC, 2020).

Exercise is commonly cited as a way to prevent and improve the symptoms of type 2 diabetes and prediabetes (Hawley & Gibala, 2012). While many of today's modern inventions and innovations have reduced poverty and increased quality of life for countless people, over the past five decades we as a society have engineered our way out of physical labor into a highly inactive civilization with lower daily expenditures and higher caloric intakes (Church et al., 2011). While exercise is certainly important, recent evidence shows that reducing physical inactivity, time spent sitting, and overall sedentary behavior is very important for diabetes prevention and maintenance. In fact, it has been recently shown that physical inactivity is considered an independent risk factor for type 2 diabetes, cardiovascular disease, stroke, and metabolic syndrome (Benjamin et al., 2018)**.** Typically,

recommendations to exercise are given to combat the detriments of physical inactivity, though regular exercise alone may not be enough to combat a lifetime of prolonged sitting day after day. Several epidemiological studies report that the major health risks of prolonged sitting are apparent even in individuals who meet the guidelines for exercise (Biswas et al., 2015; Patel et al., 2010; Young et al., 2016)**.** This evidence suggests that individuals who meet current exercise recommendations while sitting for most of their day (>10 hr/day) may still be at risk for type 2 diabetes, cardiovascular disease, and all-cause mortality (Patel et al., 2010; Proper et al., 2011)**.**

Even still, exercise has been shown to improve insulin sensitivity after periods of physical inactivity (Arciero et al., 1998; Heath et al., 1983; Rogers et al., 1990), yet in terms of postprandial plasma triglycerides, it has been demonstrated that acute exercise cannot overcome triglyceride elevating effect of sedentary lifestyles (Akins et al., 2019; Duvivier et al., 2013; Kim et al., 2016; Wolfe et al., 2020). Viewed together, when combined with sedentary behavior, there are health benefits exercise can provide, but as long as most of the day is spent in sedentary conditions, some health benefits of exercise may not be fully realized. That being said the purpose of this review is to discuss specifically the effects of physical inactivity and exercise on insulin sensitivity.

Insulin Sensitivity and Health

Insulin is a hormone that is necessary to transport glucose into cells for energy use and storage as glycogen. As postprandial blood glucose levels rise, insulin is secreted by the pancreas. The insulin binds to receptors on the cell membrane, and after a cascade of reactions, GLUT4 glucose transporters are translocated to the outer cell membrane (Cartee,

2015). Physical inactivity, poor nutrition, and other factors can cause the body to become resistant to insulin, causing the pancreas to overproduce the hormone and blood glucose levels to remain elevated, resulting in a condition known as insulin resistance. The most common cause of insulin resistance and type 2 diabetes is obesity, and the prevalence of type 2 diabetes in obese populations has been studied and accepted since the 1930's (Joslin et al., 1936). While many factors contribute to the development and exacerbation of insulin resistance, including physical inactivity, poor nutrition, and other factors, this review will focus on insulin sensitivity and physical inactivity as a key contributor to poor insulin action and insulin resistance.

Both insulin and exercise act as separate and independent pathways to redistribute GLUT4 glucose transporters from within the cell interior to the cell surface membrane, facilitating glucose transport from the blood into the cell (Cartee, 2015; Constable et al., 1988). Exercise-stimulated glucose uptake provides an alternate way for glucose clearance for insulin-resistant patients, which effects will be discussed later in this literature review.

Patients rarely develop type 2 diabetes mellitus alone. Comorbidities are common and develop alongside insulin resistance and diabetes by way of unhealthy habits and lifestyles. Iglay et al. investigated the prevalence of common comorbidities among over 1.3 million diagnosed type 2 diabetes patients. They found that 98% of patients had at least one comorbidity, and 89% had at least two. Among the patients 82% also had diagnosed hypertension, 78% were overweight/obese, 77% had elevated lipid profiles, 24% had chronic kidney disease, and 22% had cardiovascular disease (Iglay et al., 2016). Over a lifetime of sedentary behavior, excessive caloric surplus, and other unhealthy habits,

metabolic ailments develop alongside one another. It is for this reason that the American Heart Association, American College of Sports Medicine, and the American Diabetes Association list physical inactivity as a risk factor for type 2 diabetes, cardiovascular disease, and metabolic syndrome (Colberg et al., 2010; Young et al., 2016). This was done as an effort to combat the pernicious effects of sedentary behavior on metabolic and general health.

Physical Inactivity and Health

Modern advances in technology over the last fifty years have caused many more people to work in office settings doing sedentary work, leading to lower daily energy expenditures (Church et al., 2011). Sedentary activity, defined as any waking behavior with an energy expenditure ≤ 1.5 metabolic equivalents in a sitting or reclining position (Sedentary Behaviour Research Network, 2012), is associated with impaired lipid tolerance, elevated blood glucose, and insulin resistance in both animal (Bey & Hamilton, 2003) and human models (Bankoski et al., 2011; G. Healy et al., 2008; G. N. Healy et al., 2008).

In a meta-analysis published in 2012, Edwardson et al. investigated associations between sedentary behavior and metabolic syndrome. They defined metabolic syndrome as adults with central obesity and at least two comorbidities in the following categories: raised blood pressure, raised triglycerides, reduced high density lipoprotein (HDL) cholesterol, and raised fasting glucose. Gathering data from ten cross-sectional studies and over 21,000 subjects, they found that increased sedentary time elevated the risk of metabolic syndrome by 73% with no differences in subgroups of sex, income, or measure

of sedentary behavior (Edwardson et al., 2012). More epidemiological evidence examined television viewing time as a proxy for sedentary behavior. Grøntved et al. demonstrated that every 2-hour difference in television viewing time per day was associated with a 20% difference in diabetes risk, a 15% difference in cardiovascular disease risk, and 13% difference in all-cause mortality (Grøntved & Hu, 2011). Chronic sedentary behavior certainly increases risk for disease, and while regular exercise is often prescribed as a remedy, it may not be a cure-all for metabolic diseases if people remain largely sedentary for the remainder of the day outside of planned exercise.

Physical activity performed at a moderate to vigorous intensity has been shown to improve the detrimental metabolic effects of a sedentary lifestyle by way of increasing daily energy expenditure and increasing the insulin signaling pathway (Hawley & Gibala, 2012). Those who are routinely physically active are also less likely to suffer from cardiovascular disease and type 2 diabetes (Benjamin et al., 2018). In efforts to help individuals be physically active and remain healthy, The American College of Sports Medicine (Garber et al., 2011) and World Health Organization (*Global Recommendations on Physical Activity for Health*, 2010) released recommendations that to maintain good health, people should participate in at least 150 min/week of moderate to vigorous physical activity for healthy adults. However, follow-up research found that fewer than one-half of Americans actually meet the recommendation (Tucker et al., 2011). Furthermore, experimental evidence suggests a relationship between prolonged sitting and a higher risk for type 2 diabetes mellitus, all-cause, and cardiovascular disease mortality (Proper et al., 2011).

Relatively short durations of physical inactivity can have deleterious effects on insulin sensitivity. As little as one day that is spent mostly sitting can dramatically reduce insulin action in young healthy adults by 20% (Stephens et al., 2011). When physical activity in young, healthy individuals was restricted to extreme lows (264 steps) for only one day, Stephens et al. (2011) found that the whole-body rate of glucose disappearance during a hyperglycemic clamp was reduced by 39%, indicating decreased insulin sensitivity after one day of inactivity (Stephens et al., 2011). These young, healthy, untrained subjects were kept in energy balance. Many studies (Arciero et al., 1998; Henriksen, 2002; Rogers et al., 1990; Seals et al., 1984) have demonstrated that moderate exercise acutely improves insulin sensitivity and lowers plasma glucose, suggesting that exercise has a notable effect on improving insulin sensitivity.

Even trained endurance athletes experience similar effects in acute physical inactivity. Arciero et al. (1998) found that 7 – 10 days of detraining in trained endurance athletes caused a 65% and 79% increase in area under the curve for glucose and insulin, respectively (Arciero et al., 1998). Heath and Holloszy (1983) had trained subjects undergo an oral glucose tolerance test in both a trained state and after 10 days of physical inactivity. They found that after 10 days of no training, these trained individuals showed a 100% increased insulin response compared to their trained state 10 days earlier. Despite the increased insulin levels, these subjects also exhibited increased blood glucose concentrations (Heath et al., 1983). Inactivity also negatively effects glucose tolerance in older athletes. After having subjects undergo 10 consecutive days of inactivity, Rogers et al. (1990) showed that while many masters athletes during normal training conditions had

a glucose tolerance comparable to much younger athletes, after 10 days of physical inactivity their glucose tolerance was also attenuated. He also showed a significant increase in plasma insulin after the 10-day inactivity intervention, suggesting impaired insulin sensitivity (Rogers et al., 1990).

Exercise Effects on Insulin Sensitivity

If physical inactivity is a factor in diabetes and prediabetes, a logical solution is to be physically active and to perform regular exercise. Exercising is overwhelmingly regarded as a beneficial way to decrease the risk of developing diabetes, cardiovascular disease, and a host of other debilitating chronic diseases. It is thus fitting to consider the effect of exercise on insulin sensitivity. Many reports have reached this same conclusion and show that exercise improves insulin sensitivity (Cartee & Holloszy, 1990; Mikines et al., 1988; Perseghin et al., 1996; Wallberg-Henriksson et al., 1988).

Exercise increases insulin-stimulated glucose uptake by the skeletal muscle. Investigating this phenomenon, Richter et al. (1989) had subjects undergo single-leg knee extensions while measuring insulin-stimulated glucose uptake in both exercising and non-exercising limbs. They found greater insulin-stimulated glucose uptake in the exercised muscle around 3-10 hours post exercise (Richter et al., 1989). Using labeled glucose molecules, Perseghin et al. (1996) tested glucose uptake during a hyperglycemic hyperinsulinemic clamp. They found that exercise increased insulin-stimulated muscle glucose uptake 48 hours after exercise compared to non-exercised conditions (Perseghin et al., 1996). Taken together, the findings of these studies suggest that the effects of acute exercise on skeletal muscle glucose uptake last long after the exercise is completed, though

some research suggest that acute exercise after periods of physical inactivity may blunt the cardiometabolic effects and adaptations to exercise.

One of the first studies to investigate the effects of inactivity on the benefits of exercise was done published by Heath et al. (1983). The investigators had young, healthy, trained subjects (at least 45 min/day training 5-7 days/week for the preceding 6 months) undergo an oral glucose tolerance test (OGTT) before spending 10 days refrain from any exercise training. On the 11th day, the subjects underwent a second OGTT. On the afternoon of the 12th day, the subjects performed a bout of exercise comparable in duration and intensity to their normal training. A third OGTT was performed on the morning on the 13th day. The three OGTTs allowed the investigators to compare the subject's glucose tolerance in their normal, trained state, after having spent 10 days without training, and then again after one training bout. They found that 10 days of physical inactivity decreased glucose tolerance, and that a single bout of exercise returned glucose tolerance almost to the same level as in the trained state. While 10 days of inactivity raised plasma glucose concentrations 10-25%, plasma insulin concentrations increased 55-120%. A single bout of exercise returned the insulin concentrations close to the first OGTT, though they remained elevated (Heath et al., 1983). These findings suggest that while exercise has beneficial effects on glucose tolerance and insulin action, physical inactivity may blunt those effects.

Recent research suggests that shorter bouts of exercise spread out throughout the day may improve insulin action. Using subjects that were overweight or obese, Larsen et al. (2019) had subjects undergo nine three-minute bouts of simple resistance exercise

consisting of bodyweight squats, calf raises, and single-leg knee raises every 30 minutes for five hours and compared it to a non-exercise control. They found that the exercise group's glucose did not change, though the postprandial insulin concentrations were attenuated by the simple resistance exercise and the insulin incremental area under the curve decreased by 26% and was significantly lower than the non-exercise control (Larsen et al., 2019). Furthermore, other researchers have reported that breaking up sitting with low-intensity activity may be more beneficial to glucose tolerance and insulin action than moderate exercise. Duvivier et al. (2013) had 18 healthy subjects complete three conditions for four days each, consisting of sitting 13 hours/day with one hour of vigorous exercise, breaking up sitting with walking and standing, and a completely sedentary control, each trial ending in an OGTT. They found that one hour of exercise did not improve insulin sensitivity or plasma lipids when subjects spent the rest of the day sitting, though when subjects broke up sitting with minimal-intensity physical activity, measures of insulin sensitivity, insulin area under the curve, and plasma triglycerides all decreased (Duvivier et al., 2013). Duvivier et al. (2017) studied these effects further in type 2 diabetic patients. Using a very similar protocol, they found that breaking up sitting with standing and light-intensity walking improved 24-hour glucose and measures of insulin sensitivity, while sitting for 13 hours with an hour of exercise failed to improve 24-hour glucose or measures of insulin sensitivity (Duvivier et al., 2017).

Acute exercise has powerful effects on the already insulin resistant. In insulin resistant obese rats, Betts et al. demonstrated that intermittent exercise improved insulin responsiveness and net glucose uptake up to 48 hours after intermittent exercise (Betts et

al., 1993). Another group demonstrated similar findings in a different breed of rat (Gao et al., 1994). Studies in human subjects demonstrate similar findings. Devlin and Horton investigated insulin action in both obese and lean men 12 hours after a bout of glycogen-depleting exercise and compared it to a non-exercise control. They found that acute exercise increased insulin-stimulated rates of glucose disposal in the obese subjects for at least 12-14 hours. The lean subjects showed small increases, though they were not statistically significant. While both groups showed improvements in insulin action, the insulin-stimulated glucose uptake was not equalized between the groups (Devlin & Horton, 1985). Later studies comparing insulin action between insulin resistant and healthy groups corroborated these findings (Castorena et al., 2014; Perseghin et al., 1996), suggesting that while acute exercise may not equalize insulin resistant and healthy groups to the same levels of insulin-stimulated glucose disposal, it certainly improves insulin action for the already insulin resistant. Exercise is vital for combatting insulin resistance, though recent evidence suggests that regular exercise alone may not be enough to stave of metabolic ailments.

Exercise Following Physical Inactivity

Recent studies from the Human Performance Laboratory at the University of Texas at Austin have investigated the effects of prolonged sitting on the cardiometabolic benefits of acute exercise and showed that physical inactivity may blunt some of the metabolic benefits of exercise. Trombold et al. (2013) had subjects undergo three different conditions, including a high-intensity exercise bout, a moderate exercise bout, and a non-exercise control. Subjects performed the exercise in the evening, and the next morning were

administered a high-fat tolerance test, which consisted of baseline triglyceride, glucose, and insulin levels, then monitoring those for six hours following the ingestion of a high-fat, high-carbohydrate milkshake. The investigators found that both intensities of exercise improved the lipid tolerance of the subjects compared to the non-exercise control, suggesting that exercise has beneficial effects on lipid tolerance (Trombold et al., 2013). Kim et al. (2014) directly investigated the effect of prolonged sitting on the triglyceride-lowering effect of acute exercise. After having subjects undergo a four-day intervention of prolonged sitting, an hour of running at a moderate intensity failed to improve lipid tolerance (Kim et al., 2016). Akins et al (2019) also investigated the effect of physical inactivity on the cardiometabolic effects of exercise, and showed that an hour of running after four days of physical inactivity (< 4,000 steps) failed to improve lipid tolerance or glucose tolerance when compared to a controlled, non-exercise trial (Akins et al., 2019). While each of these studies from the Human Performance Laboratory measured hourly glucose and insulin values over the course of the high-fat tolerance test, no true measure of insulin action was undergone with an oral glucose tolerance test, which would allow for direct measurement of insulin action. Another common thread in the aforementioned studies from the Human Performance Laboratory is the well-controlled conditions for physical inactivity and the standardization of exercise intensity.

Purpose and Hypothesis

The purpose of this proposed study is to investigate the acute effect of prolonged sitting and a single bout of moderate-intensity exercise on postprandial concentrations of

plasma glucose and insulin. We hypothesize that cycling for 1 hour at 65% peak oxygen consumption (VO_{2peak}) after taking fewer than 4,000 steps (SIT+EX) will not be different in postprandial plasma insulin responses compared to the control of only sitting (SIT). Furthermore, we hypothesize that the SIT and SIT+EX groups will have a less favorable insulin response compared to a physically active trial (taking > 11,000 steps) after performing the same 1-hour exercise bout (ACTIVE+EX).

Methodology

Research Participants

Five healthy, untrained to recreationally active subjects (male (n=2) and female (n=3)) were recruited to participate in this study. Participant characteristics are noted in Table 1. Participation criteria required no history of cardiovascular or metabolic diseases, consumption of medications known to alter metabolism, and being physically able to perform the required exercise bouts. Participants were given written and verbal explanation of the study design, requirements, and risks associated with the study. Informed consent was obtained only after the participants showed clear understanding of the study requirements and risks. The Institutional Review Board of the University of Texas at Austin approved the study design and consent. This study was registered at www.clinicaltrials.gov with NCT04195165 as the identifier.

Research Protocol

Each participant completed three trials in a randomized, crossover design. Each trial occurred over five days with a washout period of at least three days between each trial. The first two days of each trial served as a control period for familiarization and control

for physical activity levels for the subsequent intervention days. Following each control period, participants performed one of three interventions. The interventions consisted of varying step counts and exercise, including fewer than 4,000 steps and no exercise (SIT), fewer than 4,000 steps with one hour of moderate cycling (SIT+EX), or more than 10,000 steps with one hour of moderate cycling (ACTIVE+EX).

Preliminary Testing

Before performing the trials, each participant came to the laboratory for initial testing. To complete this testing, participants visited the Human Performance Laboratory (HPL) for a 8-12 min exercise test to measure peak oxygen consumption while cycling (VO_{2peak}).

Controlled Activity Phase

The controlled activity phase consisted of the two days prior to each intervention. During the Controlled Activity Phase, participants were asked to follow their typical dietary and activity routine while refraining from exercise. While the participants were not limited in their step counts during this phase, they were asked to monitor their steps using a method of their choosing, including various smartwatches or an application on their smartphone. On the second day of the Controlled Activity Phase, subjects came into the HPL to be fitted with an activity monitor (activPAL) on their right thigh. The activity monitor is a small, flat, non-invasive device that contains an inclinometer and accelerometer which allows for proper measurement of body position, step count, and estimated energy expenditure. It was placed inside a waterproof cover and secured to the participant using Tegaderm.

Intervention Phase

The intervention phase consisted of the two days after the Controlled Activity Phase. Participants were asked to follow their typical dietary routine for all trials and to eat the same meal for dinner on the second day of the Intervention Phase. During the SIT trial, participants were asked to take fewer than 4,000 steps in order to achieve a baseline level of inactivity. They were asked to maintain the step count for the two consecutive days.

During the SIT+EX trial, participants were also asked to take fewer than 4,000 daily steps. On the evening of the second day, participants came to the laboratory to perform one hour of cycling at 65% of VO_{2peak}. Rating of perceived exertion (RPE) was measured every five minutes using the Borg Scale (6-20). VO_2 was monitored every 10 minutes and heart rate was measured throughout the cycling bout. Gas analysis was performed using oxygen and carbon dioxide analyzers (Models S-3A/I and CD-3A, respectively; AEI Technologies, Pittsburgh, PA) and software (MOXUS Software; AEI Technologies, Pittsburgh, PA) while subjects breathed through a one-way breathing valve (Hans Rudolph).

During the ACTIVE+EX trial, participants were asked to take more than 10,000 daily steps. Like the SIT+EX trial, participants came to the laboratory on the evening of the second intervention day to perform an hour of moderate cycling as described above. Participants were asked to consume the same meal the evening before each oral glucose tolerance test for each of the three trials.

Oral Glucose Tolerance Test Phase

The morning following each intervention phase, participants arrived at the laboratory to begin the measurements of substrate oxidation, oral glucose tolerance test

(OGTT), and plasma triglyceride responses. Subjects rested in a supine position for five minutes. While remaining supine after the five-minute resting period, subjects were asked to breathe into a meteorological balloon for 10 minutes to monitor baseline measurements of substrate oxidation, which were then analyzed by mass spectroscopy (MGA 1100; Perkin-Elmer, ST Louis, MO), and a spirometer for gas volume (Vacumed, Ventura, CA) to determine whole-body fat oxidation and metabolic status. Following the expired gas sample collection, a baseline blood sample was taken, after which participants ingested a solution containing 75 grams of glucose (Trutol 75, Fisher Diagnostics). After completion of the glucose solution, blood was sampled at 15, 30, 60, 90, and 120 minutes following the protocol listed above. Substrate oxidation was again measured during the 110-120 min period.

Energy expenditure and substrate oxidation were calculated from oxygen consumption, carbon dioxide levels, and respiratory exchange ratio (RER) based on the calculations validated by Frayn (Frayn, 1983).

% Energy from carbohydrate (CHO) oxidation = ((RER – 0.707)/0.293) x 100

% Energy from fat oxidation = 100 – % Energy from CHO oxidation

CHO oxidation (kcal/min) = (%CHO oxidation/100) x VO_2) x 5.05kcal/L O_2

Fat oxidation (kcal/min) = ((1-%CHO oxidation/100) x VO_2) x 4.7kcal/L O_2

Energy expenditure (kcal/min) = CHO oxidation + Fat oxidation

Measurements and Instrumentation

Anthropometric Measurements

Participant body mass was measured on a digital scale (Ohaus, CW-ll, Parsippany, NJ) and recorded to the nearest 0.5 kg. Similarly, height was measured using a standard stadiometer and recorded to the nearest cm. Both measurements were measured before any testing during each visit to the laboratory and were also used to calculate body mass index (BMI) as a ratio of body mass (kg) to height (m) squared.

Blood Sampling and Analysis

Following a 12-hour overnight fast, blood samples were collected during the OGTT by way of catheterization of the antecubital vein. Approximately 4 ml of blood was drawn regularly as described above. All venipunctures were performed by a certified phlebotomist using aseptic techniques. The blood samples were collected into K2 EDTA tubes (BD Vacutainer, Fischer Scientific, Hampton, NH) and centrifuged at 3,000 rpm at 4°C for 10 min. Plasma was aliquoted into individual tubes and stored at -80°C until further analysis.

Glucose was measured from commercially available kits (Pointe Scientific, Inc., Canton, MI). Immediately prior to analysis, plasma samples were removed from the -80°C freezer, allowed to thaw, and homogenized. Briefly, a 5 μL sample of plasma was added to 1 mL of glucose reagent and mixed gently. Tubes were then allowed to incubate for 3 min at room temperature and immediately read via spectrophotometry (Cary Eclipse Florescence Spectrophotometer, Agilent Technologies, Santa Clara, California, USA) at 340 nm. Triglyceride concentration was measured using a similar protocol. After vortex mixing, 3.5 μL of sample was added to 350 μL triglyceride reagent (Pointe Scientific, Inc., Canton, MI) and mixed. The solution was incubated for 30 min at 37°C on an oscillating

tray. Following the incubation period, the microplate was read at 500 nm using a microplate reader (Tecan Infinite 200 Pro, Tecan Group Ltd., Mannedorf, Switzerland). Plasma insulin was measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (ALPCO, Salem, NH) using 25 μL of plasma sample and measured on a microplate reader (Tecan Infinite 200 Pro, Tecan Group Ltd., Mannedorf, Switzerland).

Physical Activity and Step Monitoring

Participant activity and step count were monitored from the start of the controlled activity phase until the completion of the OGTT. During the controlled activity phase, participants monitored their step count using a pedometer of their choice (i.e. smartwatch, smartphone, detachable pedometer, etc.). During the intervention phase, physical activity and step count were monitored continuously by a non-invasive monitor (activPAL, PAL Technologies, Glasgow, Scotland). The monitor measured approximately 2 in x 1 in x 0.1 in and was worn anteriorly on the thigh halfway between the inguinal crease and the proximal border of the patella. The device was placed inside a small, rubber sheath and attached to the thigh using a transparent film dressing. The nature of the attachment permitted bathing without removing the monitor. The activPAL contains both an accelerometer and inclinometer, and can monitor body position, steps, and intensity of activity. The steps and activity levels captured by the activPAL are not visible to the participant, so the participants were asked to monitor steps using an app on their phone or smartwatch.

Statistical Analysis

Incremental (AUC_i) and total area under the curve (AUC_T) for concentrations of plasma triglyceride, insulin and glucose were calculated. Plasma insulin, glucose, and triglyceride curves were calculated and analyzed using repeated measures two-way ANOVA (trial x time), as was daily step count. Similarly, fasting and postprandial respiratory exchange ratio (RER), as well as fat and carbohydrate oxidation, were analyzed using repeated measure two-way ANOVA (trial x time). When interactions were significant Tukey's honestly significant difference post hoc tests were run. All data were analyzed using GraphPad Prism 7 (GraphPad Software Inc., La Jolla, CA). All data are expressed as mean ± standard error of the mean (SEM). Unless otherwise noted, the level for statistical significance was set at $p \leq 0.05$.

Results

Due to the sample size (n=5), the changes in the results for the present study will primarily be expressed as percent changes. Changes that are statistically significant will also be expressed.

Participant Characteristics

Participant characteristics are outlined in Table 1. Five participants were recruited (3 females, 2 males) and each participant completed all three trials. Each participant was a healthy, untrained young adult (23.4 ± 0.8 years).

Daily Steps

Daily steps (Figure 2) were recorded. As designed, the daily step count for ACTIVE+EX was significantly higher than both SIT and SIT+EX ($p<0.01$). SIT was not statistically different from SIT+EX, all excluding the cycling exercise.

Postprandial Substrate Oxidation

Postprandial substrate oxidation is shown in Figure 3.. Each trial showed significant increases in RER (Figure 3A) from Pre to Post during the OGTT ($p<0.0001$). Fasting whole-body fat oxidation (Figure 3B) in ACTIVE+EX was 11.1% higher when compared to SIT and 23.3% higher when compared to SIT+EX. Postprandial whole-body fat oxidation in ACTIVE+EX was 53.2% higher when compared to SIT and 26.3% higher when compared to SIT+EX.

Postprandial Plasma Glucose Response

Plasma glucose concentrations were analyzed as total area under the curve (AUC_T) and incremental area under the curve (AUCi), as well as at each time point in all trials. Fasting plasma glucose was significantly lower in ACTIVE+EX when compared to SIT and SIT+EX ($p<0.05$). Neither glucose AUC_T nor AUC_i were significantly different between trials. A graphical representation of the plasma glucose responses (A), AUC_T (B), and AUC_i (C) may be seen in Figure 4.

Postprandial Plasma Insulin Response

Plasma insulin concentrations as well as total area under the curve (AUC_T) and incremental area under the curve (AUC_i) are shown in Figure 5. No significant interactions were found between trials at any time point. Compared to SIT, SIT+EX elicited a 39% lower AUC_T ($p=0.19$) whereas ACTIVE+EX displayed a 21% lower AUC_T compared to SIT ($p=0.49$). SIT+EX elicited a 30% lower AUC_T compared to ACTIVE+EX ($p=0.36$).

Compared to SIT, SIT+EX elicited a 44% lower insulin AUC_i ($p=0.21$) whereas ACTIVE+EX displayed a 19% lower AUC_i compared to SIT ($p=0.63$). SIT+EX elicited a

30% lower AUC_i compared to ACTIVE+EX (p=0.33), suggesting that activity is not a prerequisite for gaining the insulin lowering benefits of acute exercise. Thus, exercise, whether performed during SIT+EX or ACTIVE+EX demonstrated a 19-44% reduction in insulin AUC_i respectively. Graphical representation of the plasma insulin responses (A), AUC_T (B), and AUC_i (C) may be seen in Figure 5.

Postprandial Plasma Triglyceride Response

Plasma triglyceride concentrations were analyzed at each time point in each trial, as well as total area under the curve (AUC_T) and incremental area under the curve (AUCi) (Figure 6). While no significant interactions in plasma triglyceride concentration were found between trials at any time point, ACTIVE+EX elicited a 34% lower fasting plasma triglyceride concentration compared to SIT and a 19% lower triglyceride concentration than SIT+EX. Triglyceride AUC_T was 28% lower and significantly lower in the ACTIVE+EX trial than the SIT+EX trial (p=0.03), but not the SIT trial (p=0.11). Compared to SIT, ACTIVE+EX elicited a 26% lower AUC_T. Compared to SIT+EX, ACTIVE+EX elicited a 28% reduction in AUC_T.

Triglyceride AUCi was not statistically significant between trials. Compared to SIT, ACTIVE+EX elicited a 25% lower AUC_i. Compared to SIT+EX, ACTIVE+EX elicited a 23% reduction in AUC_i. A graphical representation of the plasma triglyceride responses (A), AUC_T (B), and AUCi (C) may be seen in Figure 6.

Discussion

The primary purpose of this study was to investigate the effect of physical inactivity and physical activity followed by a single bout of moderate-intensity exercise on

postprandial concentrations of plasma glucose, insulin, and triglyceride, as well as fat oxidation, during an oral glucose tolerance test. By conducting three randomized, crossover trials consisting of taking fewer than 4,000 steps (SIT), taking fewer than 4,000 steps followed by an hour of moderate exercise (SIT+EX), and taking more than 11,000 steps throughout the day followed by the same hour of moderate exercise (ACTIVE+EX). We hypothesized that cycling for 1 hour of exercise at 65% peak oxygen consumption (VO_{2peak}) after taking fewer than 4,000 steps (SIT+EX) will not be different in postprandial plasma insulin responses compared to the control of only sitting (SIT). Furthermore, we hypothesize that the SIT and SIT+EX groups will have a less favorable insulin response compared to a physically active trial (taking > 11,000 steps) after performing the same 1-hour exercise bout (ACTIVE+EX).

One of the main findings of the present study was that compared to SIT, an hour of cycling at 65% of VO_{2peak} in both exercise trials lowered plasma insulin concentration AUC_i by 44% in SIT+EX and 19% in ACTIVE+EX. These data suggest that the one-hour exercise bout tended to lower the insulin response during the OGTT after conditions of both physical inactivity (<4,000 steps) and high background physical activity (>11,000). The influence of acute exercise in lowering plasma insulin agrees with Heath et al. who had similar results in highly-trained subjects who underwent three separate OGTTs in a trained state, after ten days of detraining, and then again after one bout of exercise. After ten days of no exercise, the insulin sensitivity in these individuals was attenuated compared to their trained state, and only one bout of exercise returned insulin nearly to trained levels (Heath et al., 1983). While Heath et al. studied well-trained athletes and did not control

non-exercise physical activity during the ten-day intervention period, they found that a single bout of exercise acutely improves insulin sensitivity. The present study suggests that a one-hour bout of moderate cycling at 65% of VO_{2peak} improves insulin sensitivity regardless of the prior day's background level of physical inactivity or activity.

One possible cause of enhanced insulin sensitivity after acute exercise is reduced muscle glycogen concentration. The present study had subjects exercise for one hour at 65% of VO_{2peak}. Richter et al (1989) had subjects perform an hour of single leg extensions at 75% of maximum work capacity and measured muscle glycogen before, immediately after, and four hours after completion of exercise. The exercise decreased muscle glycogen 37% and increased insulin sensitivity (Richter et al., 1989). Bogardus et al. showed similar glycogen reduction having subjects perform short cycling intervals (45 min total), which also improved insulin sensitivity (Bogardus et al., 1983). In the present study, the exercise bouts, whether performed during SIT+EX or ACTIVE+EX demonstrated a 19-44% reduction in plasma insulin AUC_i. These findings suggest that the glycogen-lowering effects of exercise may be enough to gain the insulin-lowering benefits of exercise regardless of whether physical activity or inactivity is engaged in the previous day.

This study also demonstrated that a two-day period of physical inactivity may be sufficient to elevate fasting plasma triglyceride levels and keep them elevated throughout the two-hour OGTT, as measured by AUC_T. The plasma triglyceride AUC_i was not different in any of the trials because the plasma triglycerides decreased below the baseline triglyceride measurement in each of the trials, though the percent changes in AUC_i between trials were very similar to the percent changes in AUC_T.

In studies of postprandial plasma triglyceride concentration, Kim et al. (2016) found that after a two-day intervention of physical inactivity, an hour of exercise failed to lower postprandial triglyceride concentration as measured during a high-fat tolerance test (Kim et al., 2016). In the present study, taking fewer than 4,000 steps elicited an elevated plasma triglyceride AUC_T in both SIT (26%) and SIT+EX (28%) compared to ACTIVE+EX during the OGTT and the exercise bout did not overcome the sitting. Previous studies investigating the metabolic effects of inactivity and exercise were designed to have intervention periods of differing lengths. A selection of these studies highlight inactivity interventions of two weeks (Krogh-Madsen et al., 2010), ten days (Arciero et al., 1998; Rogers et al., 1990), four days (Akins et al., 2019). The present study also demonstrated that in order for acute exercise to increase postprandial whole-body fat oxidation, higher background physical activity (i.e. 11,000 steps) is necessary. This is consistent with the findings of Kim et al., who also reported that acute exercise failed to increase whole-body fat oxidation after four days of physical inactivity (Kim et al., 2016).

In terms of plasma triglyceride concentration, the data in the present study suggest that physical inactivity attenuated the triglyceride-lowering effects of exercise. Comparing SIT and SIT+EX shows minimal differences in plasma triglyceride AUC_T (2%) and AUC_i (3%), demonstrating that when background physical activity is low (< 4,000 daily steps), acute exercise had a minimal effect on lowering plasma triglyceride. When background physical activity is high (>11,000 daily steps) ACTIVE+EX had 28% lower plasma triglyceride AUC_T than SIT+EX, demonstrating the triglyceride-lowering benefit of exercise may be contingent upon the prior day's background level of physical activity. As

the only difference between SIT+EX and ACTIVE+EX was the daily step count, the SIT+EX trial exhibited attenuation of the triglyceride-lowering effects of acute exercise, a condition known as "exercise resistance," and has been shown to be induced by physical inactivity by several of our recent studies (Akins et al., 2019; Kim et al., 2016; Wolfe et al., 2020).

While physical inactivity induced resistance to the triglyceride-lowering effects of exercise, it seems that the insulin-lowering effects of an hour of moderate exercise are present regardless of the physical activity levels (daily steps) taken the previous day. Compared to our non-exercise inactive control (SIT), an hour of cycling at 65% of VO_{2peak} showed a 19-43% reduction in plasma insulin AUC_i in SIT+EX and ACTIVE+EX, respectively. These data indicate that there was not exercise resistance as far as the exercise-induced benefits on postprandial insulin concentrations.

The present study is not without limitations. The COVID-19 epidemic of 2020 interrupted the data collection for the study, which limited the subject count to five subjects even though the Institutional Review Board proposal initially planned for twelve total subjects. This setback certainly prevented certain percent differences from having sufficient statistical power.

In order to appropriately test the present study's findings, more subjects must be recruited and tested. As more subjects are added to the current study, the potential for a Type II error will decrease, statistical power will increase, and a clearer picture should emerge. Future research investigating the effects of physical inactivity and exercise on insulin sensitivity may investigate different intensities or modalities of exercise and their

potential effects on insulin sensitivity and fat metabolism. Future research should also investigate the timing of the exercise stimulus. The present study placed the exercise bout the evening before the oral glucose tolerance test, though differing time placement may affect the changes in insulin sensitivity and fat metabolism.

In conclusion, these data suggest that an hour of cycling at 65% of VO_{2peak} may improve the next day's plasma insulin response by 19-43% after two days of either physical activity (i.e.; >11,000 steps) or physical inactivity (i.e.; < 4,000 steps), respectively. Therefore, the insulin lowering effect of physical activity appears influenced more by a single bout of moderate-intensity exercise (i.e.; 65% VO_{2peak}) than by the number of daily background steps. On the other hand, the acute plasma triglyceride lowing effect does not appear to be influenced by acute exercise or intensity, but rather by the level of physical activity as judged by daily steps taken. These data also suggest that physical inactivity may augment the fasting and postprandial levels of plasma triglyceride concentration and attenuate whole-body fat oxidation. It thus appears that insulin sensitivity is improved by prolonged moderate intensity exercise whereas postprandial lipemia is improved by elevating daily step count.

Tables

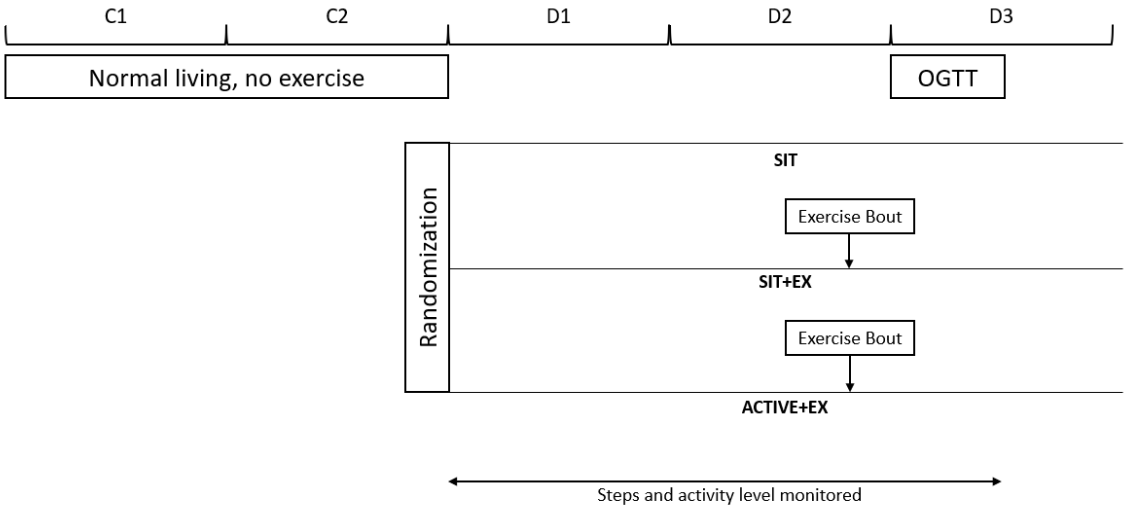
Table 1. Participant Characteristics

Characteristic	Mean ± SEM
Age (y)	23.4 ± 0.8
Height (cm)	172.0 ± 6.1
Body Mass (kg)	68.3 ± 6.9
BMI ($kg \cdot m^2$)	22.8 ± 0.9

Data are expressed are mean ± SEM.

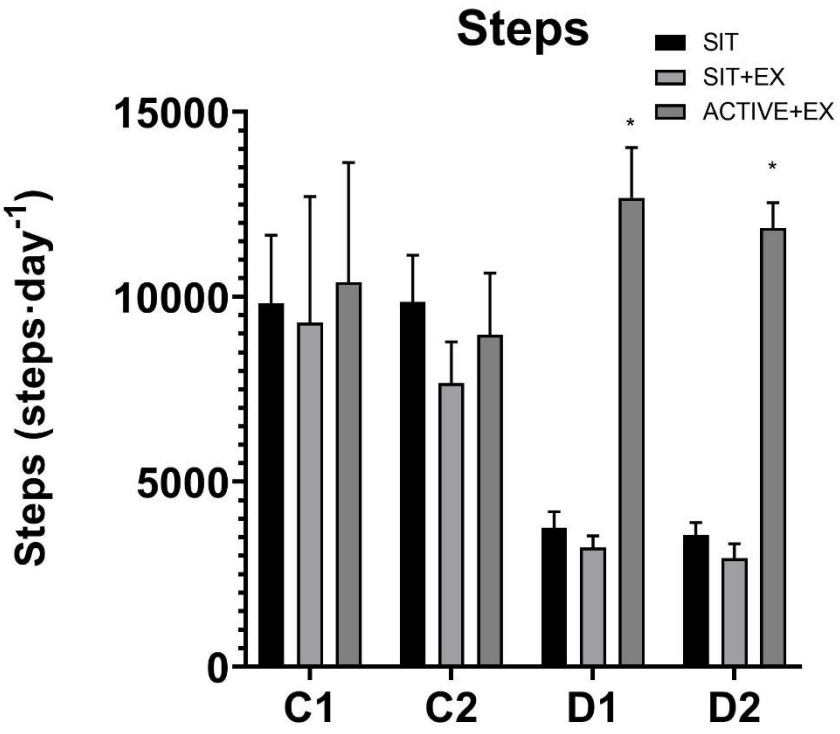
Figures

Figure 1. Diagram of the experimental protocol for all three interventions



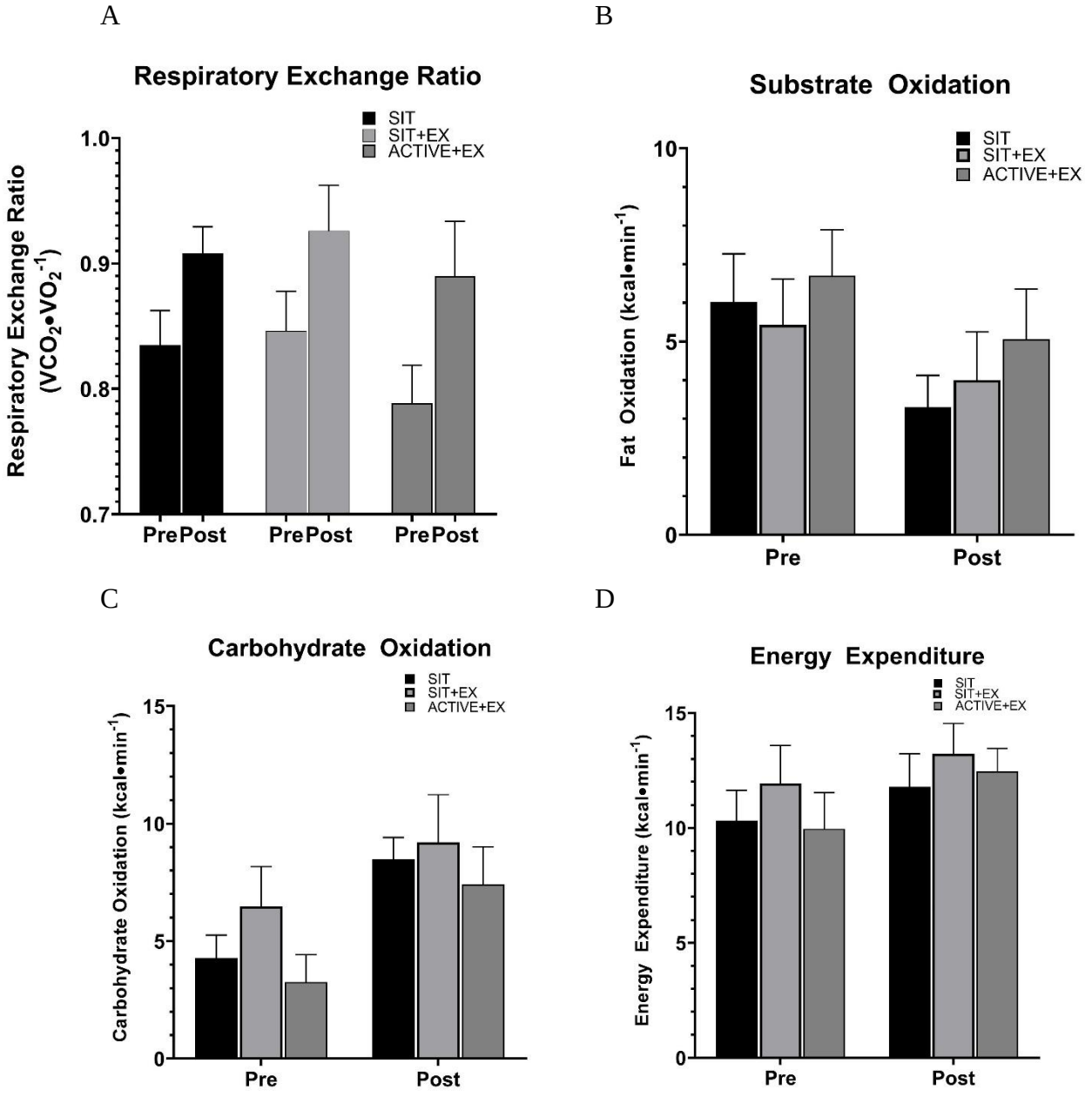
Control days (C1 and C2) and intervention days (D1 and D2). On D2, participants completed another day of inactivity (SIT), completed the same level of inactivity along with one hour of cycling at 65% of VO_{2peak} (SIT+EX), or completed another day of physical activity with one hour of cycling at 65% of VO_{2peak} (ACTIVE+EX). On D3 during all trials, each participant underwent an oral glucose tolerance test (OGTT).

Figure 2. Average daily steps



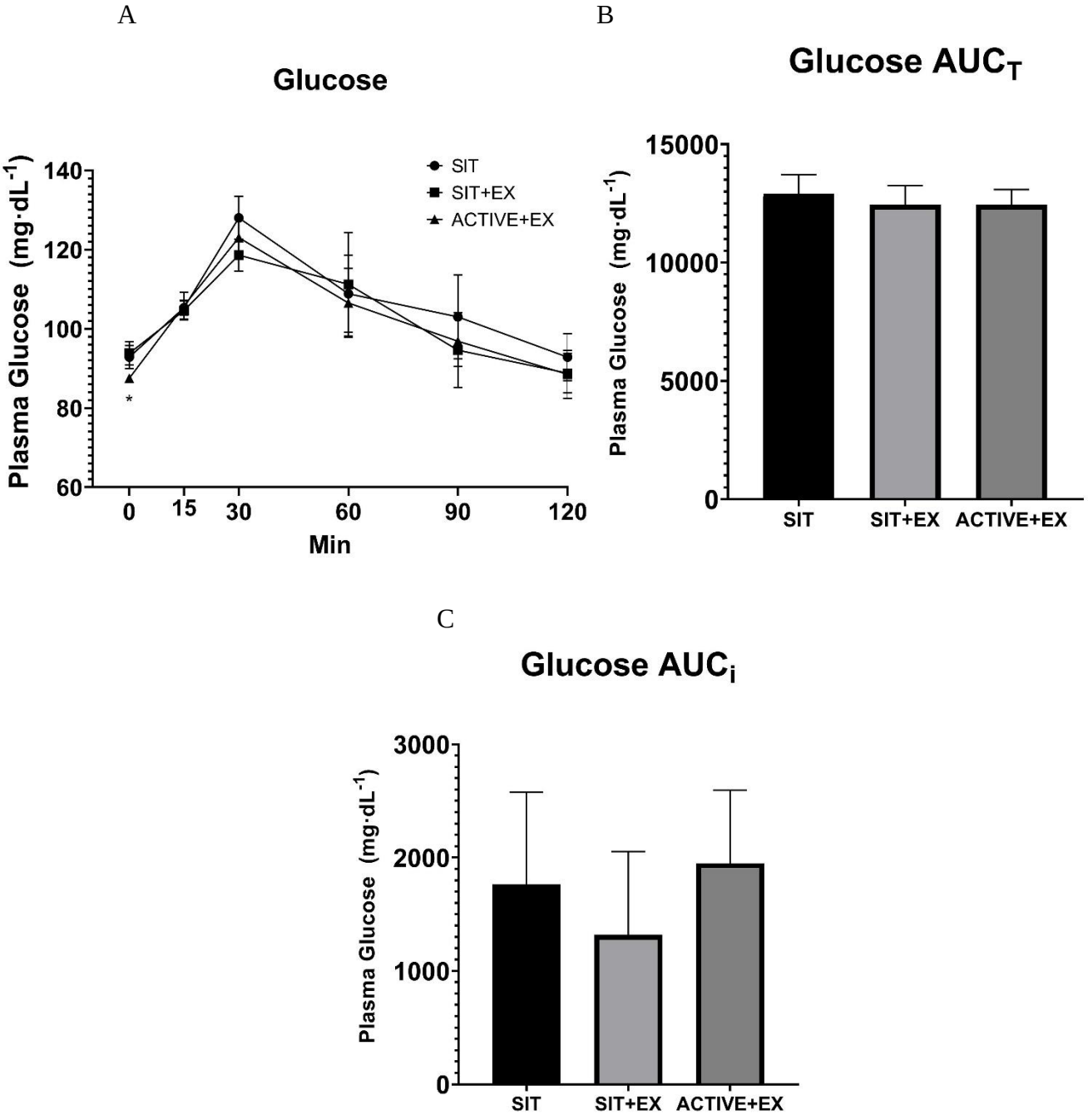
Data are expressed are mean ± SEM. (*) ACTIVE+EX significantly different from SIT and SIT+EX trials ($p<0.01$).

Figure 3. Postprandial substrate oxidation during OGTT



Respiratory Exchange Ratio (A), Percent Fat Oxidation (B), Percent Carbohydrate Oxidation (C), Energy Expenditure (D). No significant differences were found between the trials.

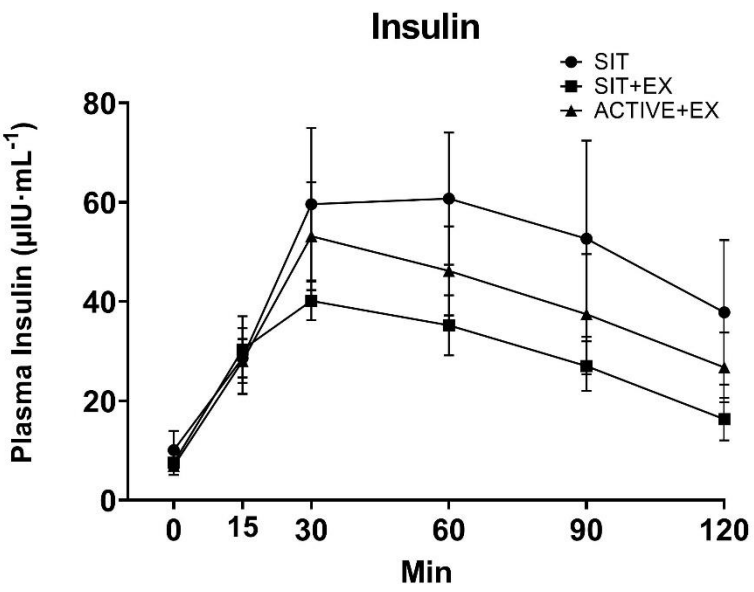
Figure 4. Postprandial plasma glucose responses during the OGTT



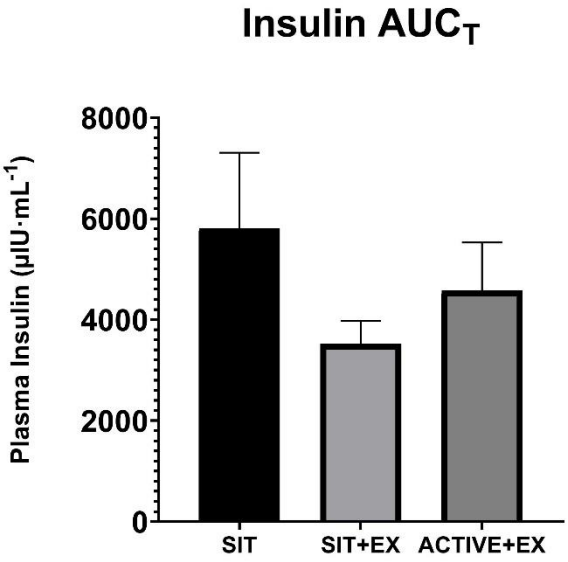
Glucose curve (A), total area under the curve (B), and incremental area under the curve (C). (*) ACTIVE+EX baseline significantly lower than SIT and SIT+EX trials (p=0.042).

Figure 5. Postprandial plasma insulin response during the OGTT

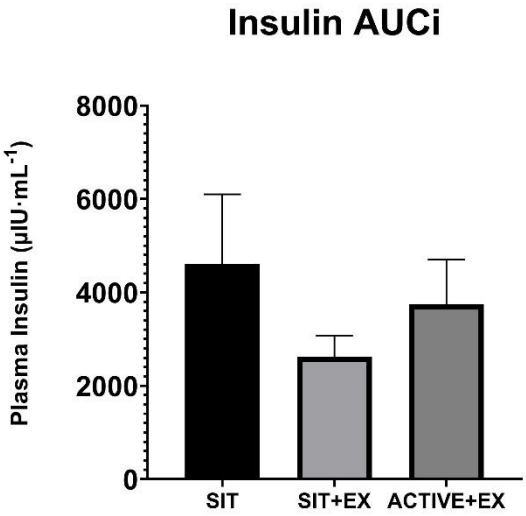
A



B

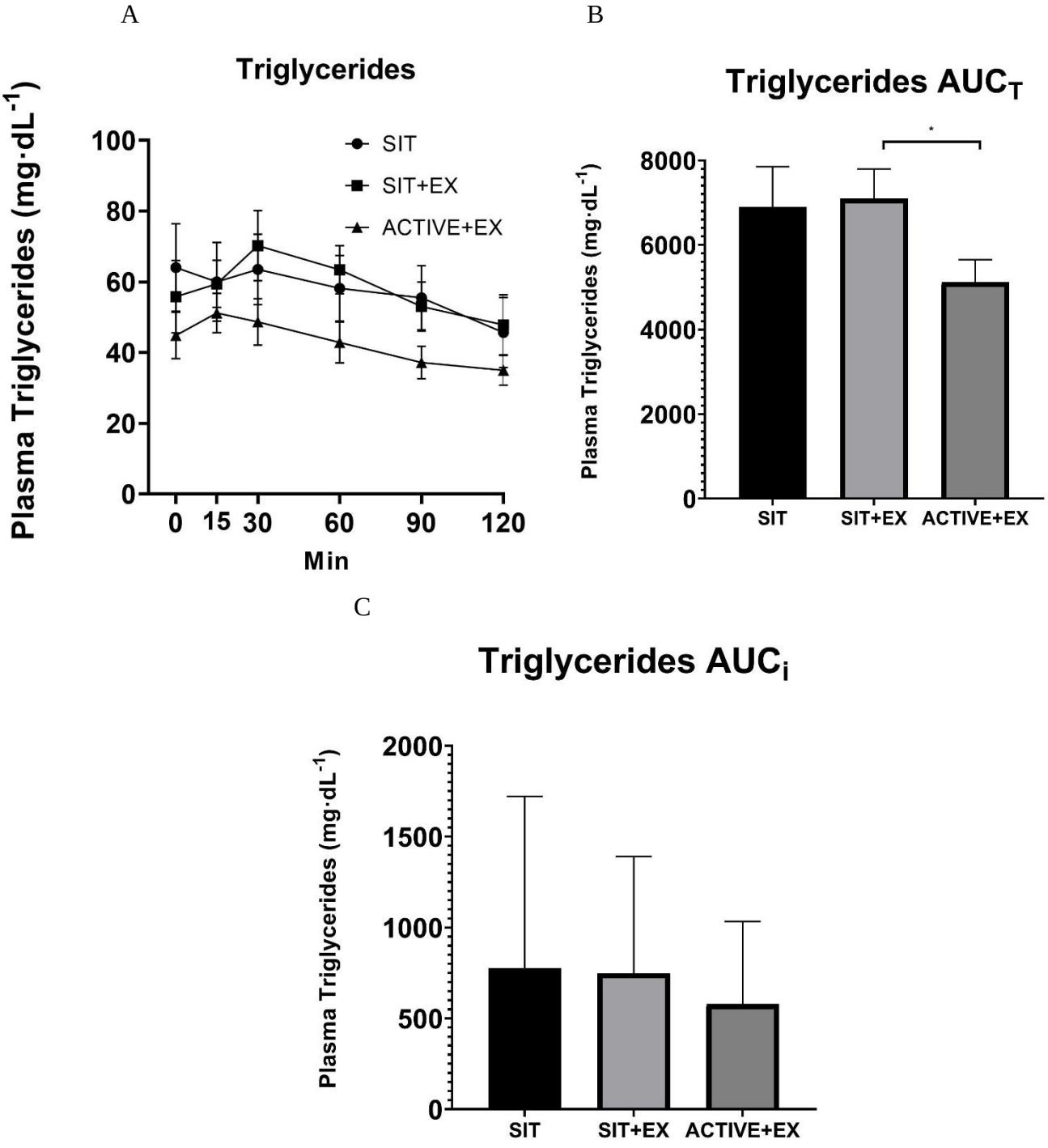


C



Glucose curve (A), total area under the curve (B), and incremental area under the curve (C). No significant differences were found between the trials.

Figure 6. Postprandial plasma triglyceride response during the OGTT



Triglyceride curve (A), total area under the curve (B), and incremental area under the curve (C). (*) Significant difference between SIT+EX and ACTIVE+EX trials (p=0.026).

Appendix A: Informed Consent

Consent for Participation in Research

TEXAS
The University of Texas at Austin

UT Austin IRB Approved
Protocol Number:
Approved:

Title of the Project: The Effect of Sitting and Moderate Exercise on Plasma Insulin and Glucose Responses to an Oral Glucose Tolerance Test
Principal Investigator: Michael Dial, Human Performance Laboratory, Department of Kinesiology and Health Education
Faculty Advisor: Edward Coyle PhD, Professor, Director of Human Performance Laboratory, Department of Kinesiology and Health Education

Consent to Participate in Research

Invitation to be Part of a Research Study

You are invited to be part of a research study. This consent form will help you choose whether or not to participate in the study. Feel free to ask if anything is not clear in this consent form.

Important Information about this Research Study

Things you should know:

- The purpose of the study is to investigate the effects of prolonged sitting and exercise on plasma insulin responses to an oral glucose tolerance test.
- In order to participate, you must be healthy and above the age of 18.
- If you choose to participate, you will perform a maximal oxygen consumption cycling test lasting 6-10 min. Then you will be asked to wear an activity monitor for three different 5-day periods. Part of these interventions involve:
- a) sitting for 13 hours for 2 consecutive days;
- b) sitting for 13 hours for 2 days with a cycling exercise bout in the afternoon of Day 2,
- c) getting > 10,000 steps for 2 days with an exercise bout in the afternoon of Day 2
- In the morning of the third day, each trial will have an oral glucose tolerance test, which involves measuring your blood glucose and insulin levels for two hours after the ingestion of a high-glucose drink. All of the testing will be performed in the Human Performance Laboratory (BEL 820).
- Risks or discomforts from this research include muscle discomfort and heavy breathing related to exercise. Possible risks associated with a venous blood sampling include bruising, light-headedness, dizziness, fainting, and rarely, infection.

- Approximately 60 ml of blood will be sampled during each of the three trials and thus the total will amount to approximately 180 ml or 6 ounces or 12 tablespoons. This amounts to approximately 2-4% of your total blood volume.
- Taking part in this research study is voluntary. You do not have to participate, and you can stop at any time.

More detailed information will be described later in this form.
Please take time to read this entire form and ask questions before deciding whether to take part in this research study.

What is the study about and why are we doing it?

In the modern world, most people pass their professional and leisure time doing sedentary activities. It has been shown that sedentary lifestyles lead to a greater incidence of heart disease, type 2 diabetes, and other negative health outcomes. It has been shown that regular exercise and physical activity can lower the risk for type 2 diabetes and cardiovascular disease. However, for many people who exercise regularly but also sit for long periods of time (~13 hrs), these major health risks are still apparent. Glucose tolerance, a predictor for type 2 diabetes, is better in those who are physically active throughout the day and impaired in those that sit for long periods of time, though many of these studies have not accounted for daily activity, namely step count. The purpose of this study is to evaluate the effect of step count, sitting time and exercise on blood glucose and insulin responses to an oral glucose tolerance test (OGTT) in twelve human subjects.

What will happen if you take part in this study?

Pretesting

If you agree to take part in this study, you will come to the Human Performance Lab (HPL) in Bellmont 820 and be asked to complete a health history questionnaire before participating. After providing informed consent, your height and weight will be measured, and you will be shown how to wear the activity monitor. We will conduct a cycling peak oxygen consumption (VO_{2peak}) test consisting of increasing cycling intensity until you are fatigued. This testing will take around 10 minutes to complete.

Trial sessions

This study contains three 4-day long trial sessions consisting of varying daily activity levels and exercise. The trial sessions are spaced out with a non-restrictive "washout" period of 2-3 days in-between the intervention weeks. The first day of each trial will be "Control" days, consisting of free living, normal physical activity levels, but no formal exercise. You will wear the activity monitor beginning on the first control day to measure physical activity throughout the trial. The physical activity conditions for the control days will be the same for all three trials. Three intervention days follow the control day and conditions differ for each. They are described in the bullet points and outlined in the table below

- SIT
    - For the first two intervention days you will take < 3,000 steps and spend ~13 hours sitting. On the morning of the third intervention day, you will come to the

HPL at 8:00 AM for the oral glucose tolerance test (OGTT) that is described below

- SIT+EX
    - For the first two intervention days you will achieve < 3,000 steps and spend ~13 hours sitting. Around 5:00 PM of the second intervention day you will come to the HPL and perform cycle exercise for 1 hour as described below. On the morning of the third intervention day you will come to the HPL at 8:00 AM for the OGTT.
- ACTIVE+EX
    - For the first two intervention days you will achieve > 10,000 steps and avoid prolonged sitting. Around 5:00 PM of the second intervention day you will come to the HPL and perform cycle exercise for 1 hour as described below. On the morning of the third intervention day you will come to the HPL at 8:00 AM for the OGTT.

	Control 1	Intervention Day 1	Intervention Day 2	Intervention Day 3
SIT	Free living	Sit ~13 hrs < 3K steps	Sit ~13 hrs < 3K steps	8 AM: OGTT
SIT+EX	Free living	Sit ~13 hrs < 3K steps	Sit ~13 hrs < 3K steps 5 PM: 1 hr cycling	8 AM: OGTT
ACTIVE+EX	Free living	Free living	Avoid prolonged sitting 10K steps 5 PM: 1 hr cycling	8 AM: OGTT

Exercise

The exercise bouts done during the two exercise trials (+EX) will be performed in the HPL at around 5:00 PM on the second intervention day. Using your pretesting data, you will cycle for 60 minutes at an intensity of 65% of $VO_{2peak,}$ which is considered moderate exercise that you should be able to maintain for over an hour.

Oral Glucose Tolerance Test

On the morning of the third intervention day you will undergo an oral glucose tolerance test (OGTT). After an overnight fast, you will come to the Human Performance Lab, have your weight measured, and drink a high-glucose drink (75 g) so we can measure your glucose and insulin responses. We will measure the responses by blood samples obtained immediately before you drink the beverage, and 10, 20, 30, 60, 90, and 120 minutes after (seven total samples). To obtain the samples, a certified phlebotomist will insert a catheter into a vein in the antecubital area of your arm. After the OGTT is completed, you will remove the activity monitor and are not restricted from doing your normal activities. The OGTT will take around 130 minutes to complete.

How long will you be in this study and how many people will be in the study?

Participation in this study will last about 4-6 weeks. Each of the three trials will take 4 days with a 3-day washout period between each trial. Pretesting will be performed one week before the first trial begins. A total of 12 individuals will participate in this study.

What risks and discomforts might you experience from being in this study?

There are some risks you might experience from being in this study. The risks associated with the maximal oxygen consumption tests include leg fatigue heavy breathing due to the intensity of maximal exercise. Possible risks associated with the blood draws include discomfort, bruising around the venipuncture site, light-headedness, dizziness, and in rare occasions, fainting or infection of the venipuncture site. The catheter will be inserted while you are lying down to minimize the risk of dizziness and/or fainting.

The researchers will let you know about any significant new findings (such as additional risks or discomforts) that might make you change your mind about participating in this study.

How could you benefit from this study?

When participating in this study, the results and interpretation from the VO_{2peak} test are provided to you, giving insight to your cardiorespiratory health.

What will happen to the samples and/or data we collect from you?

As part of this study we will collect blood samples during the oral glucose tolerance test. The samples will be analyzed for glucose, insulin, and triglycerides to examine the effect of prolonged sitting on insulin responses. All of the data will be used to examine the metabolic effect of the prolonged sitting on glucose and insulin responses to the oral glucose tolerance test.

How will we protect your information?

We will protect your information by assigning a unique Subject ID code. This informed consent form and the Health History Questionnaire are the only places where any personal identifying information will be recorded. These forms will be stored in a locked file cabinet. In all other cases, your data will only be identifiable by your unique code. Only the director of the laboratory (Dr. Coyle) will have access to a master list that will link you identify to your code.

If it becomes necessary for the Institutional Review Board to review the study records, information that can be linked to you will be protected to the extent permitted by law. Your research records will not be released without your consent unless required by law or a court order. If you choose to participate in this study, you may be photographed, or video recorded. Information about you may be given to the following organizations:

- Representatives of UT Austin and the UT Austin Institutional Review Board

What will happen to the information we collect about you after the study is over?

We might keep your research data to use for future research. Your name and other information that can directly identify you will be kept secure and stored separately from the research data collected as part of the project.

How will we compensate you for being part of the study?

You will not receive any type of payment for your participation.

Who will pay if you are hurt during the study?

If injuries occur as a result of study activity, eligible University students may be treated at the usual level of care with the usual cost for services at the Student Health Center. You and/or your insurance company or health care plan may be responsible for any charges related to research-related injuries. Compensation for an injury resulting from your participation in this research is not available from The University of Texas at Austin. You are not waiving any of your legal rights by participating in this study.

Who can profit from study results?

Your samples may be used for commercial profit and there is no plan to share those profits with you.

Your Participation in this Study is Voluntary

It is totally up to you to decide to be in this research study. Participating in this study is voluntary. Your decision to participate will not affect your relationship with The University of Texas at Austin. You will not lose any benefits or rights you already had if you decide not to participate. Even if you decide to be part of the study now, you may change your mind and stop at any time. You do not have to answer any questions you do not want to answer.

If you decide to withdraw before this study is completed, your personal information and data will be destroyed.

Is it possible that you will be asked to leave the study?

You may be asked to leave the study if it is determined by your doctor or if any of your answers to the health history questionnaire change and disqualify you from participation.

Is it safe to start the study and stop before you are finished?

You are always free to stop participating in the study if you would like. Your decision to stop participating will not affect your standard medical care or any other benefit you would receive if you were not in a research study. It is safe to stop this study before completion.

Contact Information for the Study Team

If you have any questions about this research, you may contact:
Principal Investigator: Michael Dial
Phone: 801.891.5699
Email: michael.dial@utexas.edu

Or

Faculty Sponsor: Edward Coyle
Phone: 512.471-8596
Email: coyle@austin.utexas.edu

Contact Information for Questions about Your Rights as a Research Participant

If you have questions about your rights as a research participant, or wish to obtain information, ask questions, or discuss any concerns about this study with someone other than the researcher(s), please contact the following:

The University of Texas at Austin Institutional Review Board
Phone: 512-232-1543
Email: irb@austin.utexas.edu

Please reference study number ***XXXX-XX-XXXX.***

Your Consent

By signing this document, you are agreeing to be in this study. We will give you a copy of this document for your records. We will keep a copy with the study records. If you have any questions about the study after you sign this document, you can contact the study team using the information provided above.

I understand what the study is about and my questions so far have been answered. I agree to take part in this study.

_____
Printed Subject Name

_____
Signature Date

Appendix B: Health History Questionnaire

HUMAN PERFORMANCE LABORATORY – THE UNIVERSITY OF TEXAS AT AUSTIN

IRB #: Subject ID: _____
Date of Birth (mm/dd/yy): _____ / _____ / _____ Age: _____
Height: _____ /_____cm
Weight: _____lb. / _____kg

<GENERAL HEALTH QUESTIONS>

1. Do you take any prescription or over the counter meds?
   Yes / No If yes, explain:
2. Do you have a family history of heart disease or other diseases?
   Yes/No If yes, explain:
3. Do you have any disability or impairment that affects physical performance?
   Yes / No If yes, explain:
4. Have you ever had any broken bones?
   Yes / No If yes, explain:
5. Have you ever had any major injury/surgery in lower extremities (torn major ligaments, sprain)?
   Yes / No If yes, explain:
6. Have you had any significant medical problems within the last 10 years?
   Yes / No If yes, explain:
7. Do you have any drug and/or alcohol dependence?
   Yes / No If yes, explain:
8. Do you have any heart problems or coronary artery disease?
   Yes / No If yes, explain:
9. Do you have hypertension (high blood pressure)?
   Yes / No If yes, explain:
10. Do you have any lung or respiratory problems?
    Yes / No If yes, explain:
11. Do you have type 1 or 2 diabetes?
    Yes / No
12. Do you smoke?
    Yes / No If yes, explain:
13. Do you use alcohol?
    Yes / No If yes, explain:
14. Do you use caffeine (coffee, tea, etc.)?
    Yes / No If yes, explain:
15. Do you have any allergies that require medication?
    Yes / No If yes, explain:

16. Do you experience difficulty swallowing medications or vitamins?
    Yes / No If yes, explain:
17. Do you take any dietary supplements aimed at increasing your exercise performance?
    Yes / No If yes, explain:

<SYMPTOMS ASSOCIATED WITH EXERCISE>

1. Easy fatigability or prolonged fatigue after exercise?
    Yes / No If yes, explain:
2. Persistent chest pain during and/or after exercise?
    Yes / No If yes, explain:
3. Fainting or loss of consciousness during exercise?
    Yes / No If yes, explain:
4. Palpitations (rapid, irregular, or skipped heartbeats) during exercise?
    Yes / No If yes, explain:
5. Have you ever been told to give up sports because of a health problem?
    Yes / No If yes, explain:

Appendix C: Supplemental Tables

Table 2. Average daily steps

Trial	C1	C2	D1	D2
Daily Steps (steps·day^{-1})				
SIT	9818 ± 1852	9862 ± 1265	3765 ± 427	3566 ± 337
SIT+EX	9297 ± 3494	7666 ± 1720	3219 ± 655	2936 ± 640
ACTIVE+EX	10396 ± 3239	8972 ± 1668	12678 ± 1358*	11863 ± 687*

Data are expressed are mean ± SEM. (*) ACTIVE+EX significantly different from SIT and SIT+EX trials ($p<0.01$).

Table 3. Average values for postprandial substrate oxidation

	Trial		
	SIT	SIT+EX	ACTIVE+EX
RER ($VCO_2 \cdot VO_2^{-1}$)			
Pre	0.825 ± 0.035	0.858 ± 0.159	0.788 ± 0.030
Post	0.918 ± 0.015*	0.913 ± 0.171*	0.890 ± 0.044*
% Substrate Oxidation			
Fat			
Pre	59.72 ± 8.24	49.04 ± 13.19	71.95 ± 9.93
Post	28.00 ± 5.17*	33.58 ± 10.37	43.07 ± 9.64*
Carbohydrate			
Pre	45.29 ± 7.48	50.36 ± 13.56	28.05 ± 9.93
Post	72.00 ± 5.17*	66.42 ± 14.73	56.93 ± 9.64*
Substrate Oxidation ($kcal \cdot min^{-1}$)			
Fat			
Pre	6.03 ± 1.24	5.44 ± 1.45	6.70 ± 1.19
Post	3.30 ± 0.82*	4.01 ± 1.33	5.06 ± 1.30
Carbohydrate			
Pre	4.29 ± 0.97	6.49 ± 1.92	3.25 ± 1.17
Post	8.48 ± 0.93*	9.21 ± 2.47	7.41 ± 1.61*
Energy Expenditure ($kcal \cdot min^{-1}$)			
Pre	10.33 ± 1.30	11.93 ± 2.63	9.96 ± 1.58
Post	11.78 ± 1.44	13.22 ± 2.69	12.46 ± 0.99*

Table 3. Average values for postprandial substrate oxidation. Data are expressed as mean ± SEM. (*) significantly different from Pre ($p<0.05$).

Table 4. Postprandial responses for plasma glucose, insulin, and triglyceride

Minutes Postprandial	**Trial**		
Glucose ($mg \cdot dL^{-1}$)	**SIT**	**SIT+EX**	**ACTIVE+EX**
0	94.0 ± 2.9	93.8 ± 3.0	87.4 ± 1.2
15	105.8 ± 1.9[a]	104.6 ± 2.4[a]	106.6 ± 3.4[a]
30	123.8 ± 5.4[b]	118.6 ± 4.0	121.4 ± 5.0[a]
60	101.4 ± 9.7	111.2 ± 13.1	102.0 ± 8.7
90	94.8 ± 10.6	94.6 ± 9.4	94.6 ± 6.4[c]
120	87.8 ± 5.9[c]	88.8 ± 5.0[c]	85.8 ± 6.0[c]
Insulin ($\mu IU \cdot mL^{-1}$)			
0	10.8 ± 3.9	7.5 ± 2.4	7.0 ± 0.9
15	26.9 ± 3.9[a]	30.3 ± 6.7	21.9 ± 6.6
30	45.3 ± 15.4	40.1 ± 3.9[a]	44.7 ± 10.9
60	47.7 ± 13.3	35.2 ± 6.0	38.0 ± 9.0
90	33.3 ± 19.8	27.0 ± 5.0[a]	25.8 ± 12.1
120	25.0 ± 14.6	16.3 ± 4.3[e]	21.7 ± 7.0[d]
Triglyceride ($mg \cdot dL^{-1}$)			
0	67.8 ± 12.4	55.8 ± 10.2	45.0 ± 6.5
15	62.2 ± 11.1	59.4 ± 6.7	50.6 ± 5.6
30	65.4 ± 10.0	70.2 ± 9.9	48.8 ± 6.6
60	60.0 ± 9.3	63.4 ± 6.8	42.6 ± 5.8
90	58.0 ± 9.1	53.0 ± 6.9[b]	37.6 ± 4.6
120	48.6 ± 9.9	47.8 ± 8.5[b]	35.8 ± 4.2

No significant interactions were found in trial x time. Significance across time points within trials are as follows; (a) $p<0.05$ compared to Min 0, (b) $p<0.05$ compared to Min 15, (c) $p<0.05$ compared to Min 30 (d) $p<0.05$ compared to Min 60 (e) $p<0.05$ compared to Min 90.

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